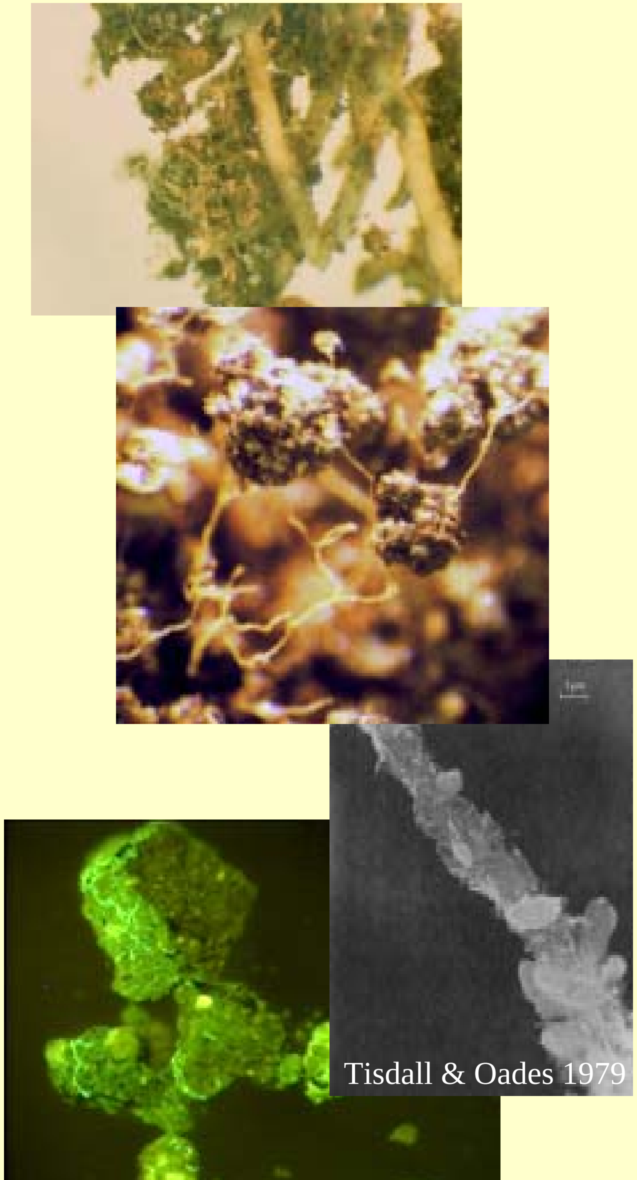

Tisdall & Oades 1979

The use of the restoration chronosequence at Fermilab to address belowground processes

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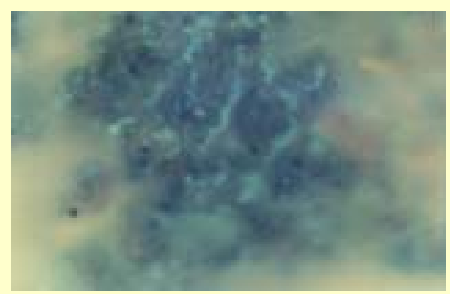


Contributors

Mike Miller (ANL) – mycorrhizal fungi
Julie Jastrow (ANL) – soil aggregation
Roser Matamala (ANL) – soil carbon
Vicki Allison (ANL) – microbial communities

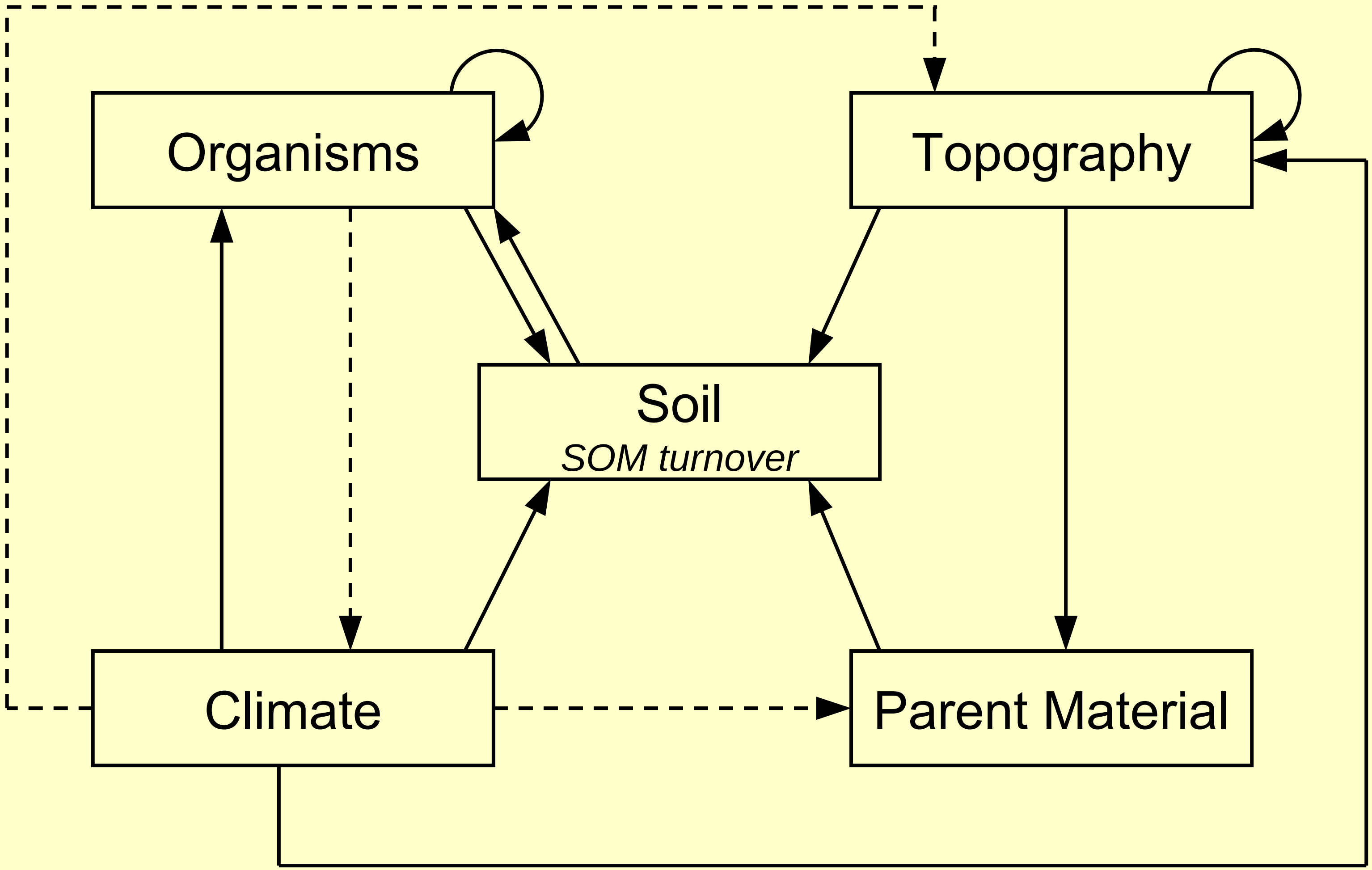
Programs
Multiple factor effects
Carbon cycling
Carbon sequestration

Research funded by:

US DOE Office of Science
Climate Change Research Division
Program for Ecosystem Research
Terrestrial Carbon Processes Program
Carbon Sequestration Program

Land use practices can significantly influence the fertility and stability of soils.

Especially affected is the amount of soil held as stable soil aggregates and their ability to sequester carbon.


Organisms
Topography
Soil
SOM turnover
Climate
Parent Material

What is soil structure?

The arrangement of particles and associated pores in soil

More modern definitions:

"The mutual arrangement of the individual soil particles, the stability of the aggregated state, and the wider range of pore sizes that result" Payne, 1988

"An arrangement of particles in soil and particles of sand, silt, and clay, bound together into aggregates of various sizes by organic and inorganic means" Tisdall, 1996

Some examples of soil stabilization by fungal hyphae

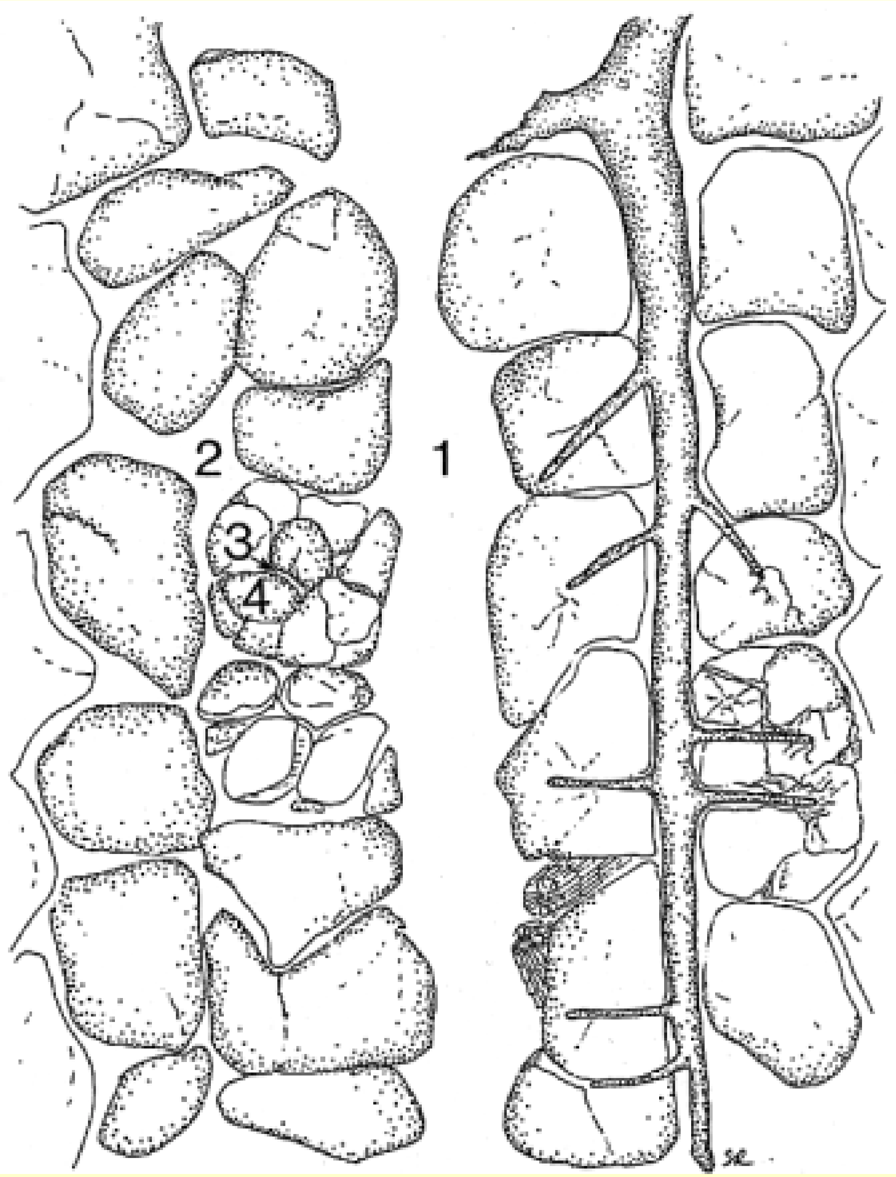
- Lichenized crusts
- Sand dune stabilization
- Soil aggregation

SOIL AGGREGATION DEFINED:

"... the naturally occurring cluster or group of soil particles in which the forces holding the particles together are much stronger than the forces between adjacent aggregates."

J. P. Martin et al. 1955. Soil aggregation. Advances in Agronomy 7:1-37.

Highly structured soils have a diversity of pore sizes created by the hierarchical organization of soil aggregates



From Elliott and Coleman, 1988, *Ecol. Bullet.* 39:23-32.
Illustrated by S.L. Rose

Functional classification of soil pores and associated soil particles (from Oades, 1984)

Pore diameters (μm)	Function	Particle diameters (μm)
<0.2	**Bound water**	**<2**
0.2-2.5	**Storage of plant-available water**	**2-250**
2.5-100	**Capillary conduction, aeration**	**250-1000**
>100	**Aeration, fast drainage, root growth**	**>1000**

Classification of pores as habitat for soil biota

(modified from Elliott and Coleman, 1988)

Pore size category	Largest organisms using each category
Intramicroaggregate (<1 μm)	Bacteria
Intermicroaggregate (within macroaggregates)	Small nematodes, protozoa, fungi, root hairs, very-fine roots
Intermacroaggregate	Nematodes, fine roots
Macropores (>1000 μm)	Microarthropods, worms, coarse roots

Soil structure ⇒ Habitable pore space

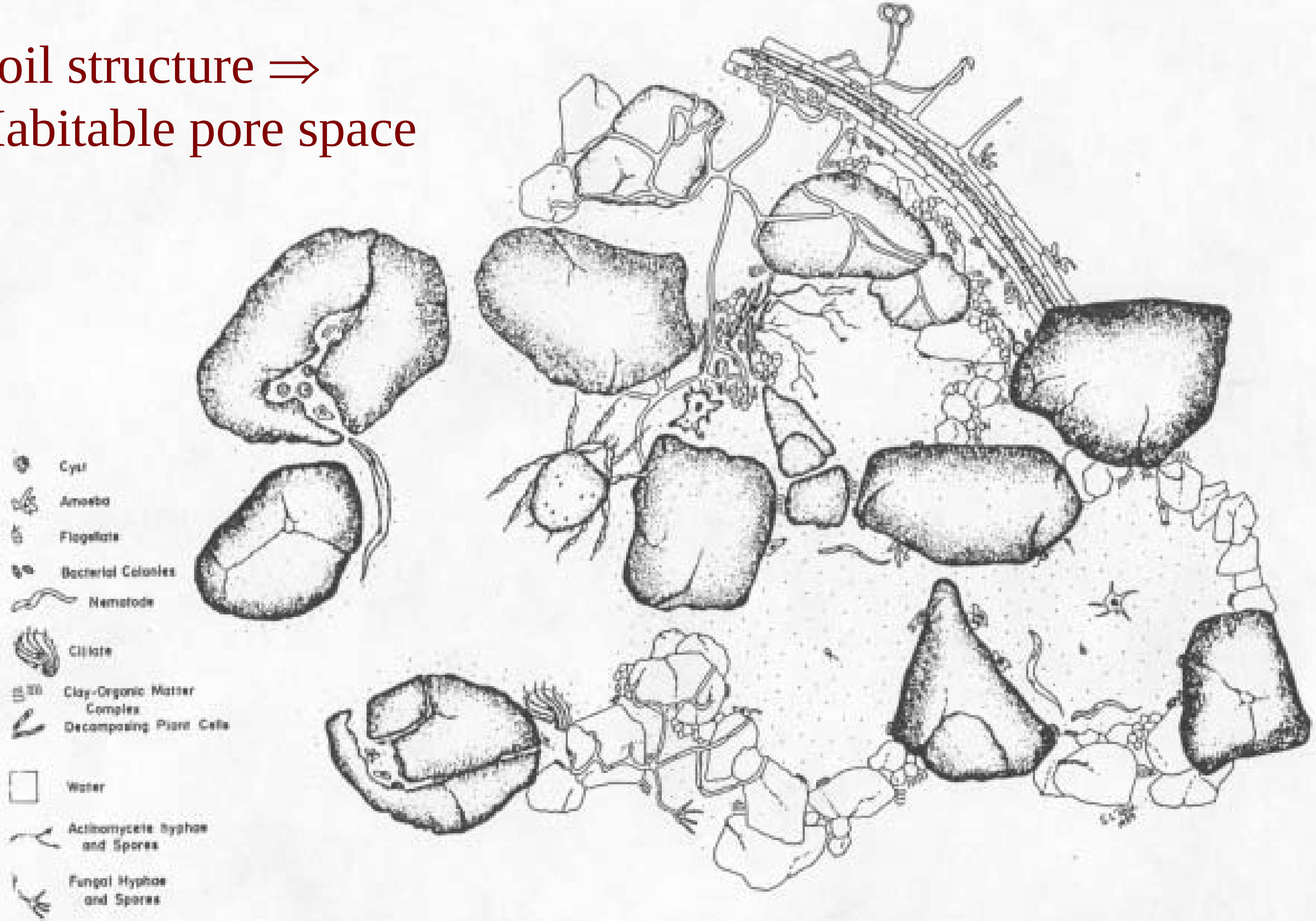


Fig. 4. Horizontal cross section ($1\ cm^2$) of a highly structured and biologically active microsite in the short grass prairie. It depicts how the different classes of pore space and the distribution of water within pores influence the feeding and habitat relationships among the different groups of soil organisms. Illustration by S. L. Rose.

Aggregate hierarchy and the porosity exclusion principle

(*Aggregates are separated from soils by disruption based on different amounts of energy*)

Larger aggregates

- *contain larger pores than smaller aggregates*
- *larger pores form planes of weakness*

Hence, smaller aggregates have

- *greater contact between particles*
- *stronger bonds between particles*
- *have a higher tensile strength*

Dexter, Soil Tillage Res 11:223-238 (1988); Oades & Waters, Aust J Soil Res 29:815-828 (1991)

Aggregate cycling

Stabilization (and Degradation)

- Physical, chemical, and electrical forces stronger than external forces
  - **Entanglement by roots and fungal hyphae**
  - **Fresh organic debris of plant, microbial, and animal origin**
  - **Organomineral associations between linear organic polymers with many active groups (extracellular polysaccharides, glomalin and other hydrophobins, some humic materials)**
  - **Clays (amounts and mineralogy)**
  - **Polyvalent metal cations (e.g., Ca^{2+}, Fe^{3+}, and Al^{3+})**
  - **Oxides of Fe, Al, Mn, and Si**
  - **Activities of decomposers (extent of protection)**

From Oades and Waters, 1991, *Aust. J. Soil Res.* 29:815-828.

The concept of soil aggregate hierarchy

Aggregation hierarchy infers a range of aggregates of different sizes and it is essential to describe precisely the scale at which structure or aggregation is being studies (nine orders of magnitude)

JM Tisdall & JM Oades, 1982, J Soil Science 33:141-163

Creation of soil structure

- Formation and stabilization of soil aggregates
  - Clays (kinds and amount)
  - Organic binding agents
    - Transient – *microbial and root exudate polysaccharides and gums that last only a few weeks*
    - Temporary – *roots and fungal hyphae that last a few months*
    - Persistent – *aromatic organic compounds linked by polyvalent cations to clay surfaces*

Tisdall & Oades, J Soil Science (1982)

Soil processes influenced by soil structure

Physical Processes

Erosion

Runoff

Infiltration

Hydraulic conductance

Fast drainage

Aeration

Habitat for Soil Biotic Processes

Nutrient Cycling

immobilization

mineralization

gaseous fixation

gaseous losses

leaching

mineral weathering

ion exchange

Carbon Cycling

respiration

carbon inputs

root turnover

MBC turnover

microbial by-products

decomposition

(aerobic v. anaerobic)

carbon accumulation

physical protection

Biological and abiotic influences on soil structure with contrasting soil textures

Property	Influence of soil type		
	Sand	**Loam**	**Clay**
Shrink-swell capacity	*minimal*	*important*	*maximum*
Abiotic aggregation	*minimal*	*important*	*maximum*
Mycorrhizal effects	*important*	*important*	*minimal*

CONCEPTUAL DIAGRAM OF AGGREGATE HIERARCHY

From Jastrow and Miller, 1998, *In* Soil Processes and the Carbon Cycle, CRC Press.

Microaggregates
~ 90-250 and 20-90 μm

Plant and fungal debris

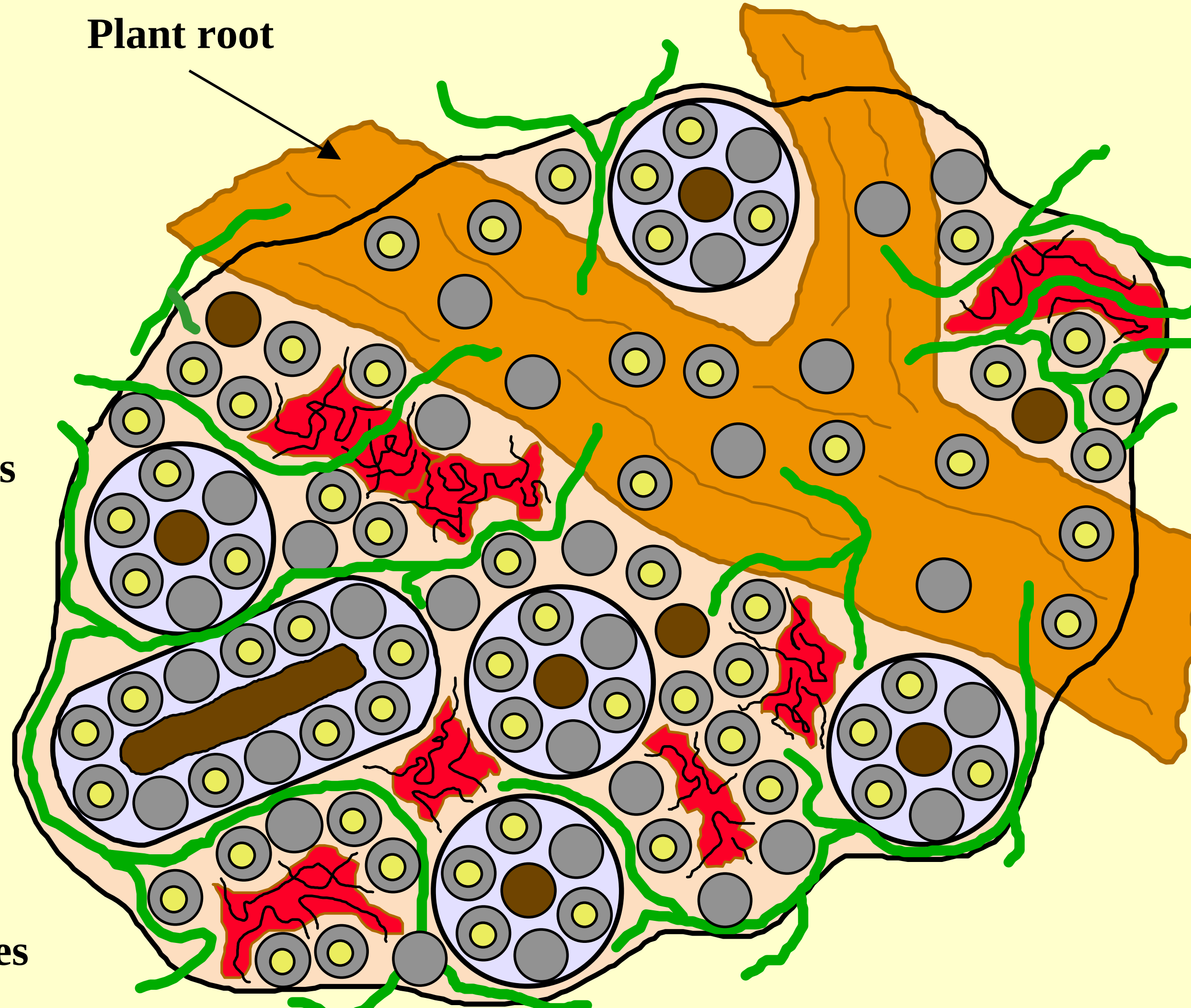
Silt-sized microaggregates with microbially derived organomineral associations

Clay microstructures

Particulate organic matter colonized by saprophytic fungi

Mycorrhizal hyphae

Pore space; polysaccharides and other amorphous interaggregate binding agents



Restoration of tallgrass prairie at Fermilab

- Restoration of tallgrass prairie began inside the accelerator ring in 1975.
- Annual plantings on similar soils have created a chronosequence of restorations.
- Restored areas (inside and outside the ring) now total over 400 ha.

- Average site precipitation of 850 mm and temperature of 8.9 C (180 days between frost).
- Vegetation is dominated by C4 grasses and perennial forbs.

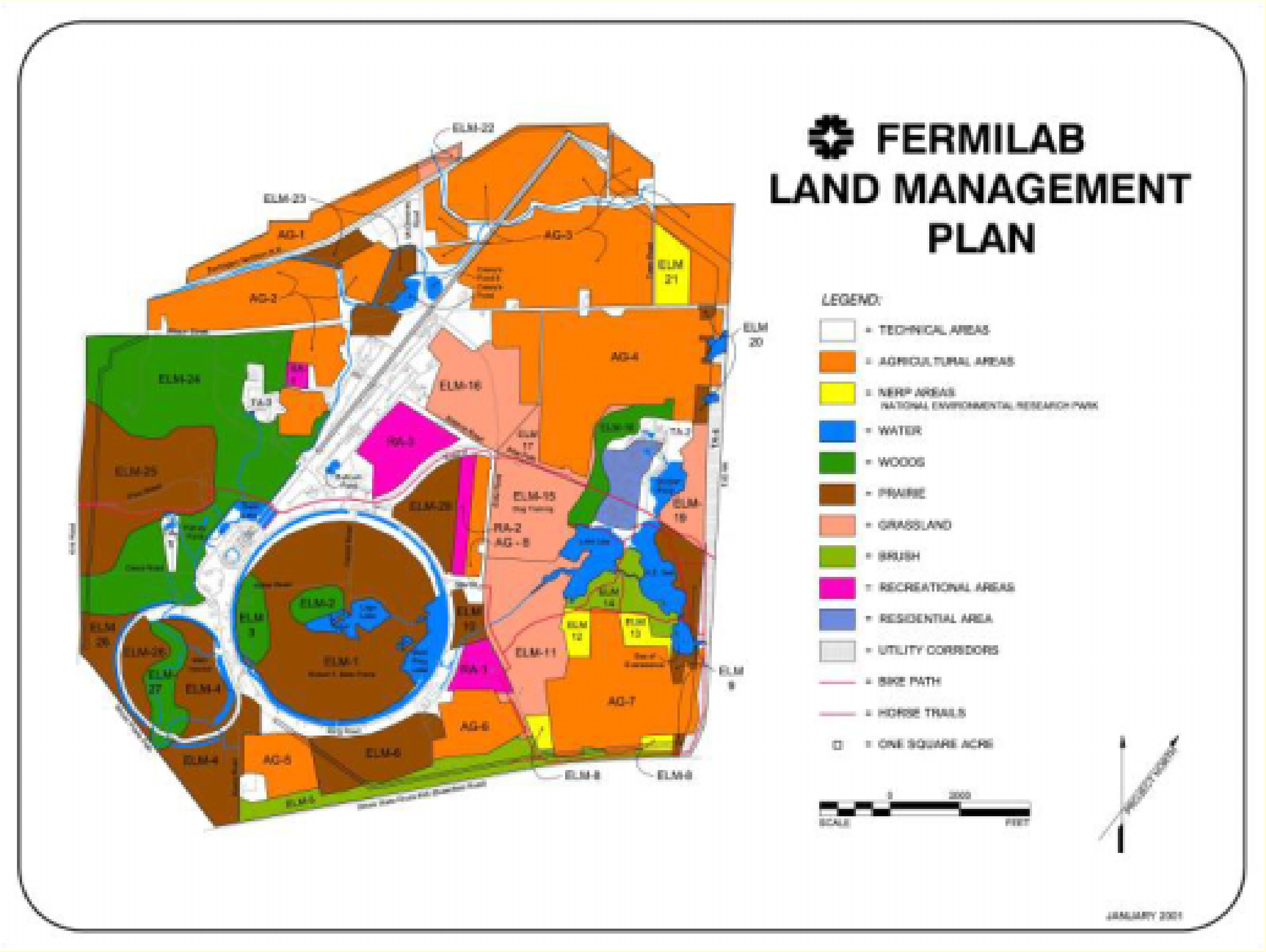
Loss of tallgrass prairie

Illinois tallgrass prairie

- 1830 87,550 km2
- 1860 10 km2

Prairie land cleared per year	3.33%
Brazil forest clearing	1.47 %
Costa Rica	1.73%
Malaysia	2.47%

Fermi Lab Restorations



Restoration tools at Fermilab

Seed harvesting

Site preparation

Planting

Fire

Fermilab Prairie Restorations

Site under cultivation since 1840’s

First year

Second year

Initial years are dominated by species typical of old-field succession (annuals → biennials → weedy perennials).

Once litter buildup is sufficient to carry a fire, prairie grasses and forbs begin to take over.

Eleventh year

Restoration at Fermilab

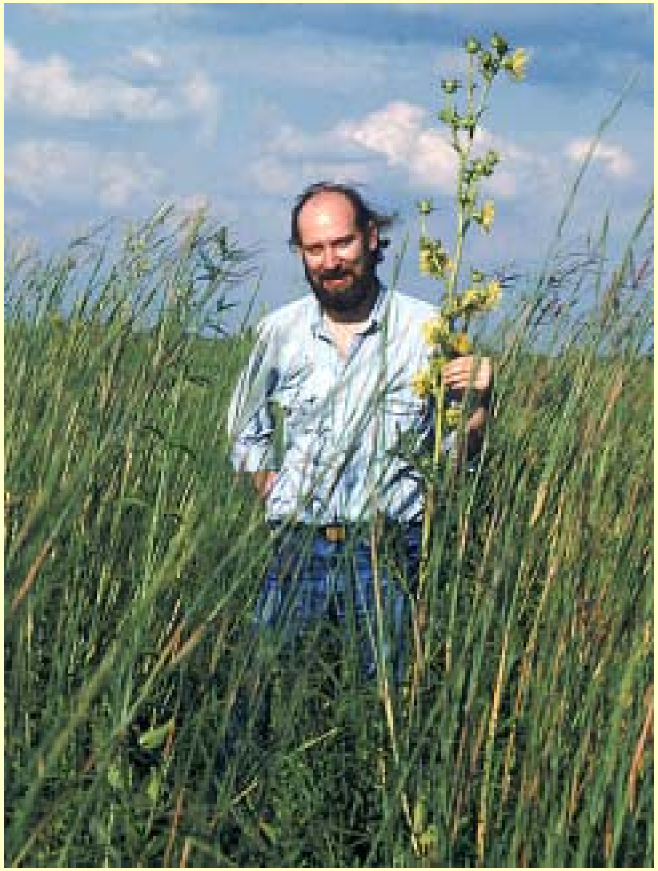
Although bison exist at Fermi they are not on the restorations

Without fire trajectory towards old field

Sweet clover can be a management problem.

Supplemental planting necessary for diversity

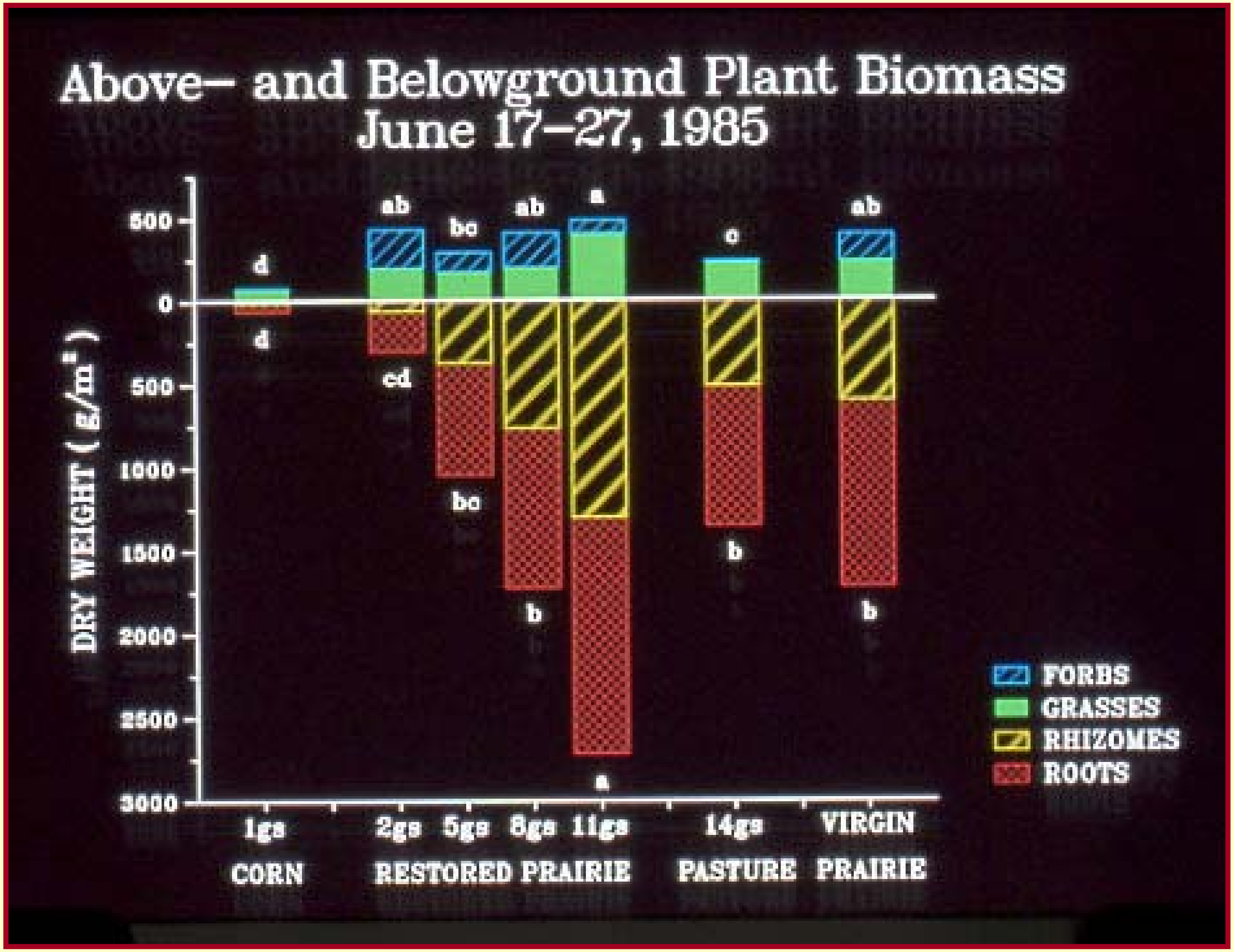
Restoration at Fermilab



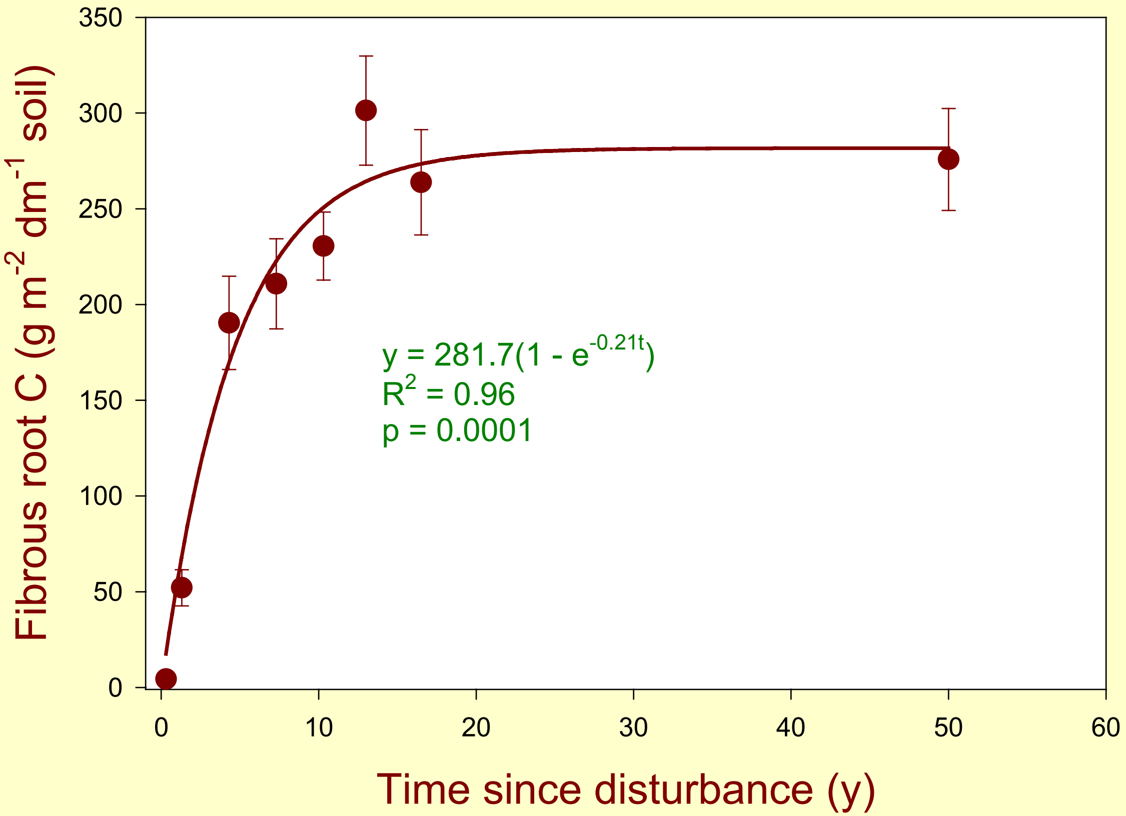
Because of yearly above ground senescence and periodic burning - peak standing crop may also a measure of above ground production.

Peak standing crops (dry wt) can be >1 kg m^{-2} aboveground and >2 kg m^{-2} belowground.

Field sampling

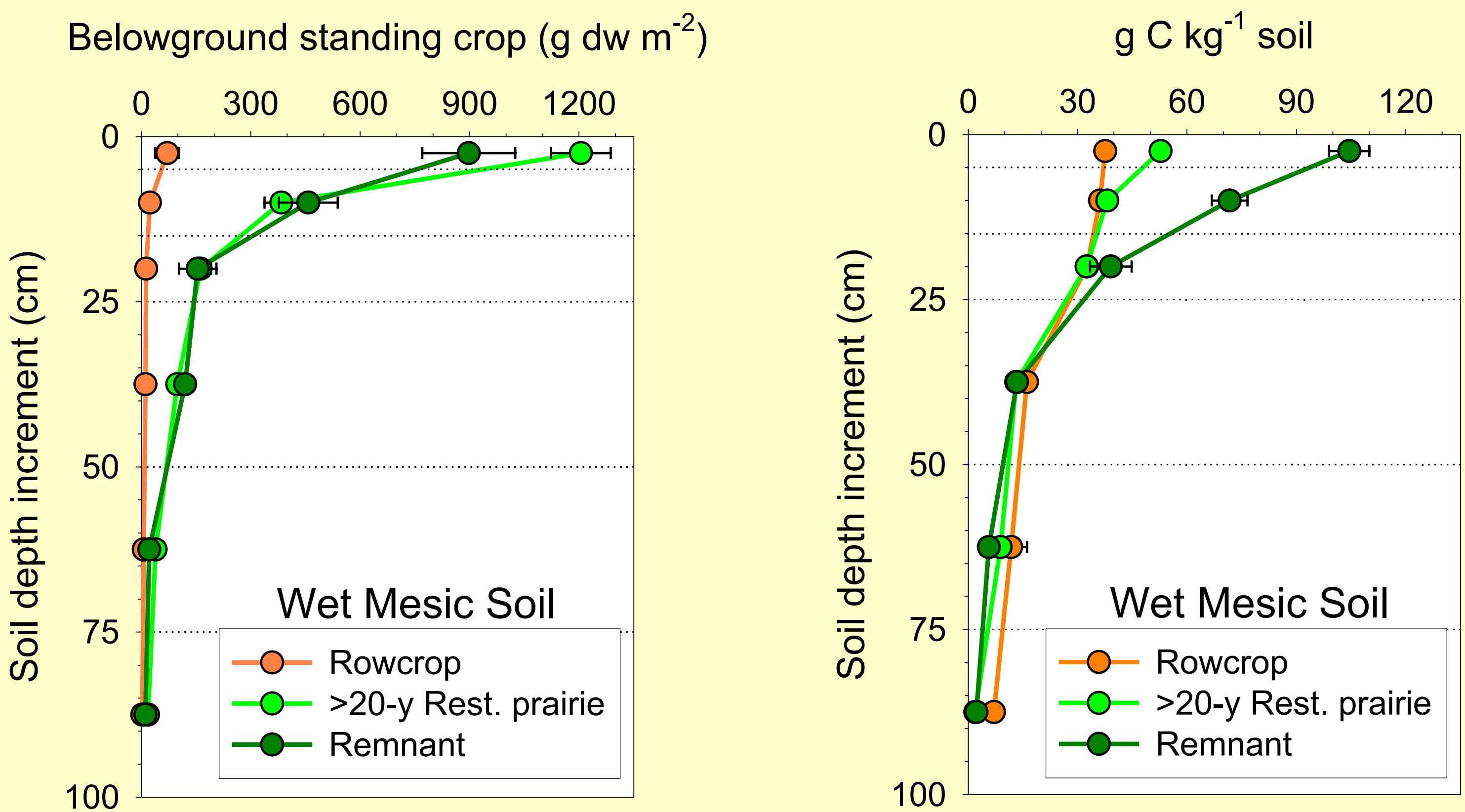

Above- and Belowground Plant Biomass
June 17-27, 1985
DRY WEIGHT (g/m²)
500
0
500
1000
1500
2000
2500
3000
d
d
ab
cd
bc
bc
ab
b
a
a
c
b
ab
b
1gs
CORN
2gs 5gs 8gs 11gs
RESTORED PRAIRIE
14gs
PASTURE
VIRGIN
PRAIRIE
FORBS
GRASSES
RHIZOMES
ROOTS

Fibrous root production along the restoration chronosequence

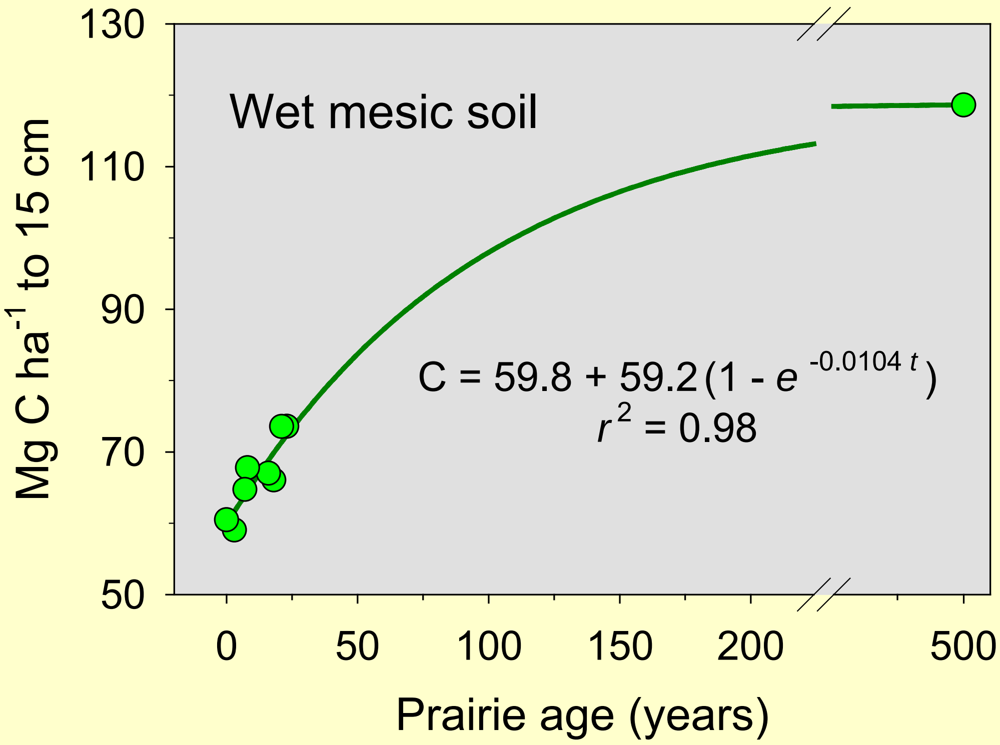


Depth distribution of inputs and soil C

- Belowground biomass in older restored prairies equals or exceeds remnants
- Root and rhizome inputs drive changes in soil C
- Greatest soil C increases in surface 5-10 cm
- Potential for long-term soil C accrual to 25-30 cm



Accrual of soil organic C sustained over 25 years



Exponential model predicts accrual of 0.54 Mg C ha^{-1} y^{-1} for 25 years in the surface 15 cm

C_e	118.6 Mg ha^{-1}
MRT	96 y
t_{50}	66 y

Based on equivalent soil mass for 0-15 cm depth at time zero

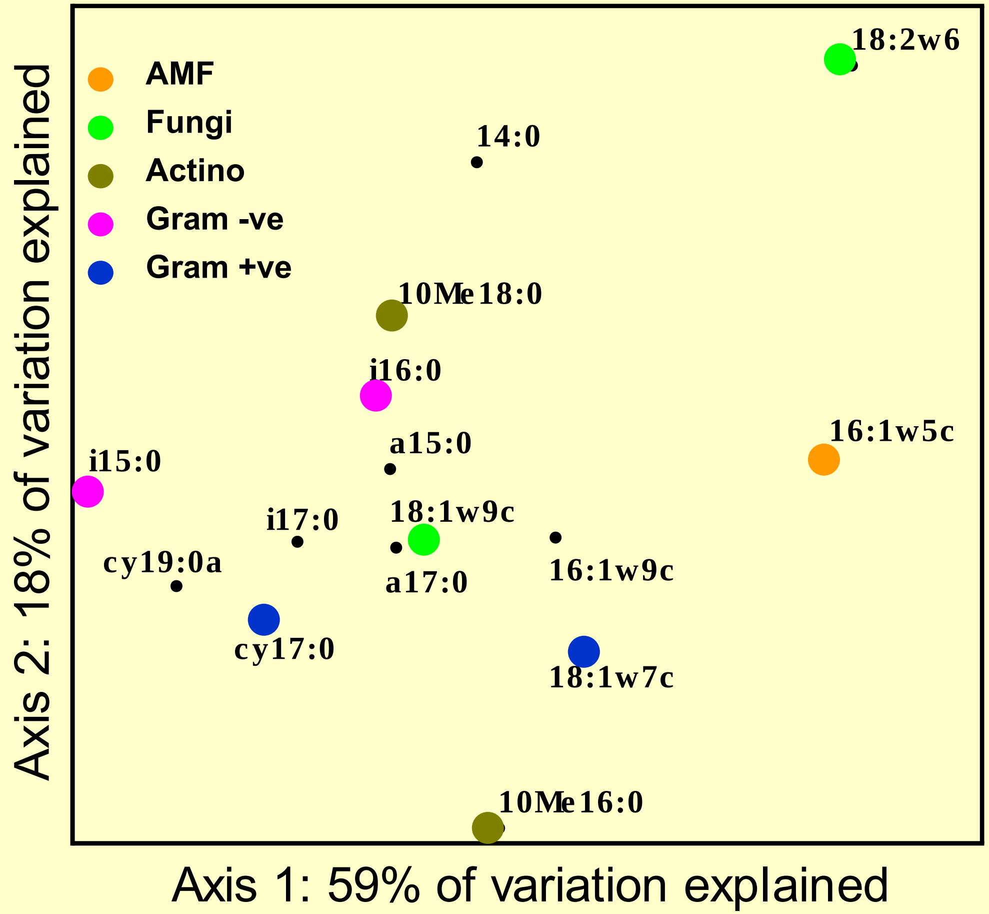
Use of phospholipids to characterize microbial community changes along the restoration chronosequence

Func. group	**Signature PLFAs**					
Actinomycetes	10Me16:0	10Me18:0				
Bacteria	14:0	a15:0	16:1ω9c	i17:0	a17:0	cy19:0
Gram +ve	cy17:0	18:1ω7c				
Gram-ve	i15:0	i16:0				
Fungi	18:2ω6	18:1ω9c				
AMF	16:1ω5c					

Fermilab Restoration Chronosequence

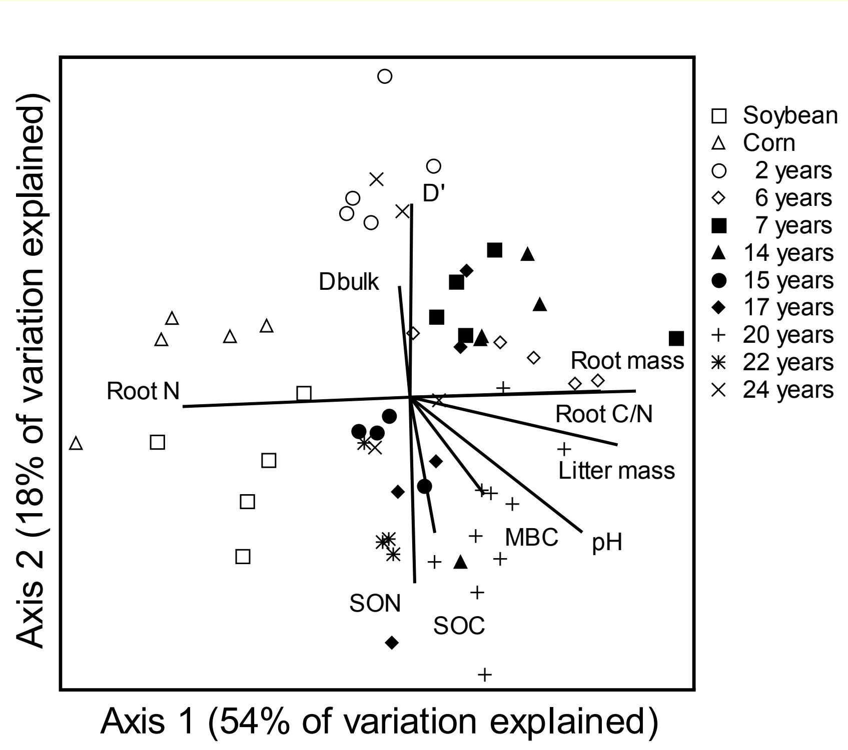
Analysis

Microbial community signature phospholipid fatty acids were summarized using reciprocal averaging (RA) analysis. The position of each sample depends on the relative abundance of 15 signature fatty acids. Sample position along each axis was subsequently related to environmental variables by linear and nonlinear regression.



Fermilab Restoration Chronosequence

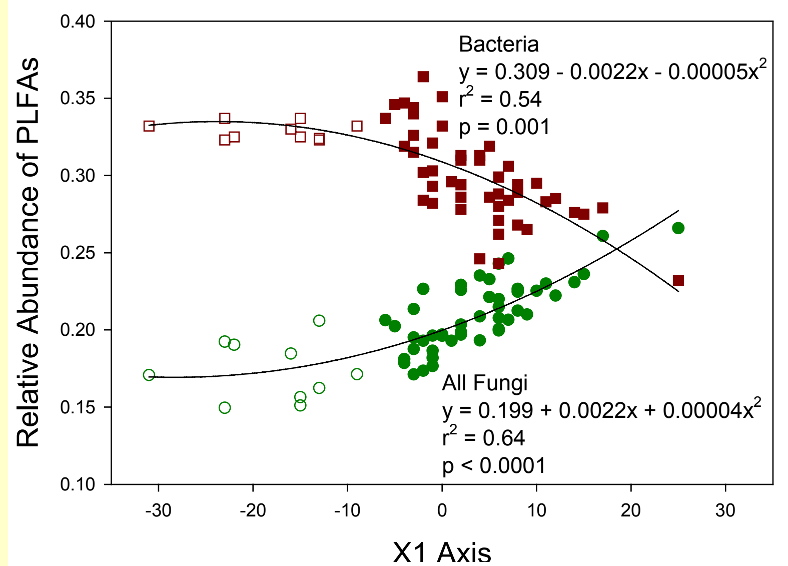
Axis 2 is most strongly correlated with soil characters, especially bulk density ($R^2 = 0.29$, $p \leq 0.0001$), pH ($R^2 = 0.31$, $p \leq 0.0001$), soil N ($R^2 = 0.42$, $p \leq 0.0001$) and soil organic C content ($R^2 = 0.30$, $p \leq 0.0001$).

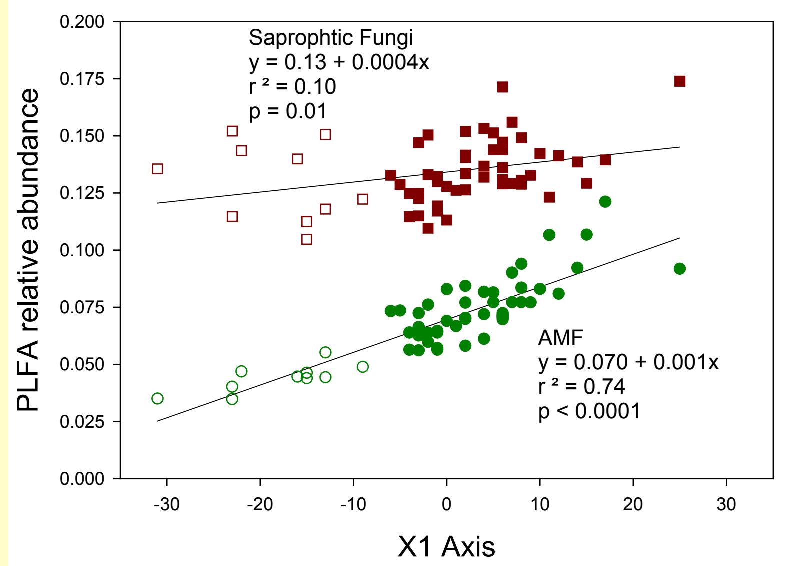


Axis 1 is most strongly correlated with changes in vegetation characters, especially root biomass ($R^2 = 0.55$, $p \leq 0.0001$), root C:N ratio ($R^2 = 0.41$, $p \leq 0.0001$), soil pH ($R^2 = 0.38$, $p \leq 0.0001$) and soil C:N ratio ($R^2 = 0.25$, $p \leq 0.0001$). Basically the left side of the graph is represented by soils agriculture and the right side by the restored prairie plots.

Fermilab Restoration Chronosequence

The major change in microbial composition is summarized by axis 1 of the ordination: this corresponds to a decline in relative abundance of bacteria, but an increase in relative abundance of fungi along the chronosequence.



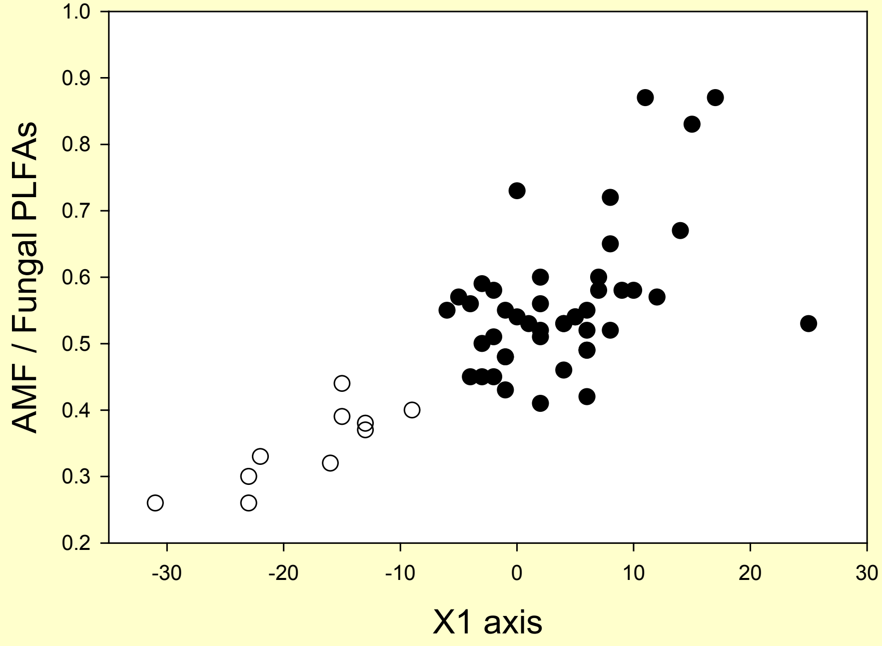


The increase in fungal PLFA relative abundance is proportionally larger for AMF than for saprophytic fungi.

Fermilab Restoration Chronosequence

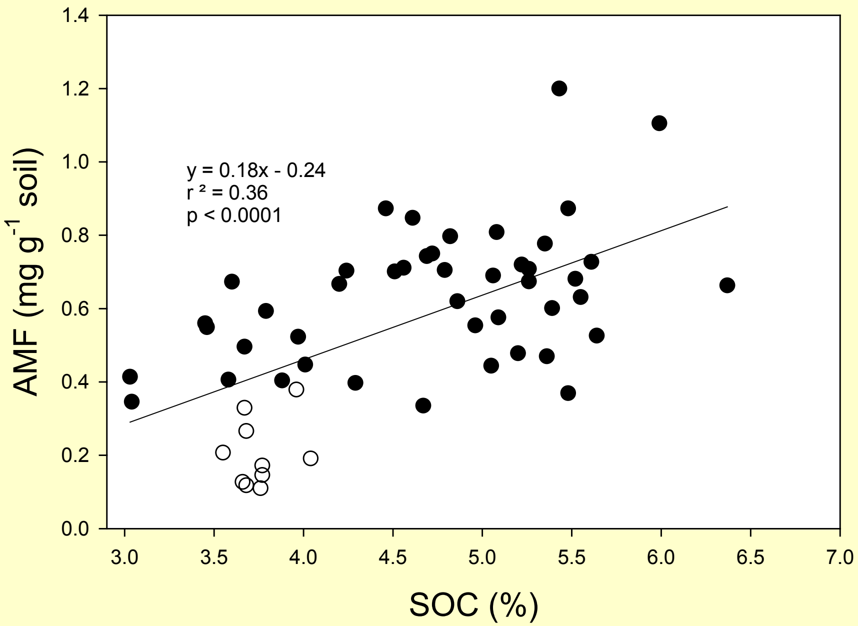
The restoration chronosequence represents a soil carbon gradient where mycorrhizal fungi become the dominant form of fungi within the system.

- a direct consequence of the development of a rhizospheric dominated soil.
- Suggests that as SOC accumulates a greater proportion of the fungal biomass will be from AMF.
- Could lead to a reduction in the efficiency of the fungal population to utilize the accumulated carbon, viz. an increase in AMF rather than saprophytic fungi (AM fungi are not able to degrade detritus carbon).



Fermilab Restoration Chronosequence

The amount of the AM fungal marker PLFA is positively associated with the accumulation of SOC within the restoration chronosequence.

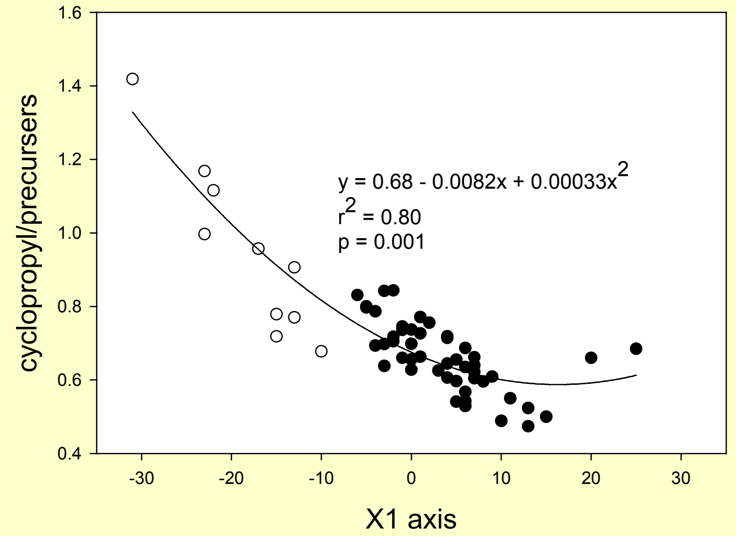


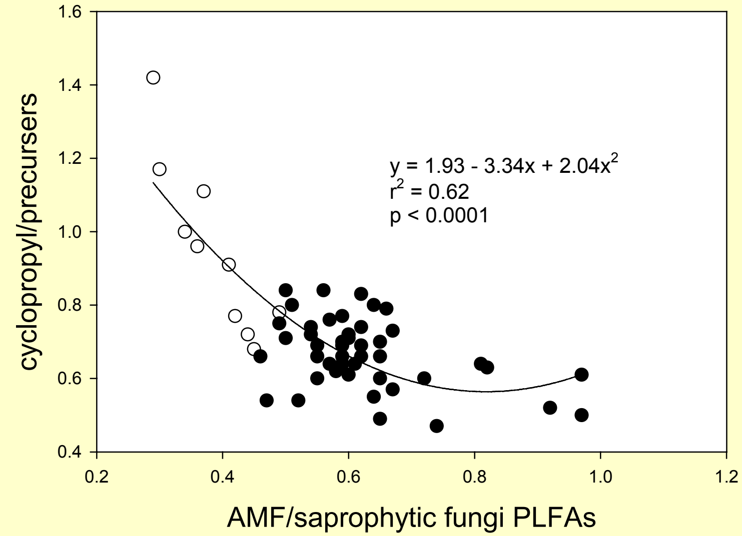
Fermilab Restoration Chronosequence

The ratio of cyclopropyl fatty acids cy17:0 and cy19:0 relative to their precursors, 16:1ω7c and 18:1ω7c, declines following conversion to prairie (represented by X1 axis).

Indicates an increase in proportion of bacterial cells in log rather than stationary phase of growth.

Suggests bacterial communities in the agricultural soils may be carbon limited.

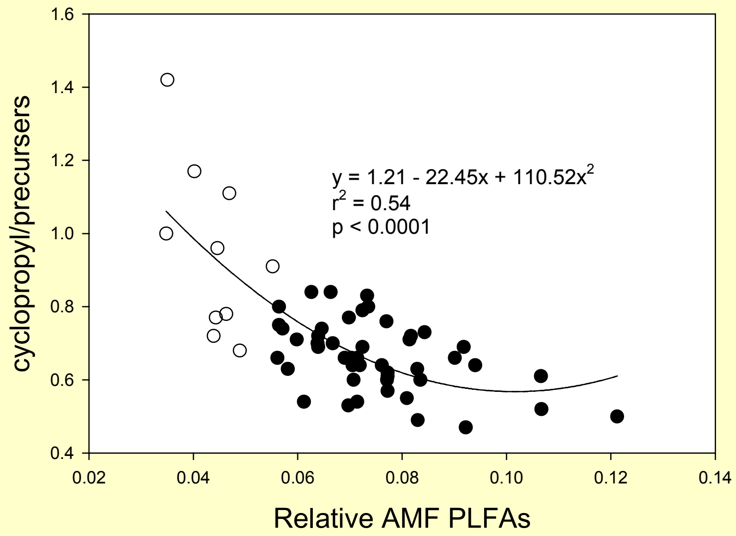




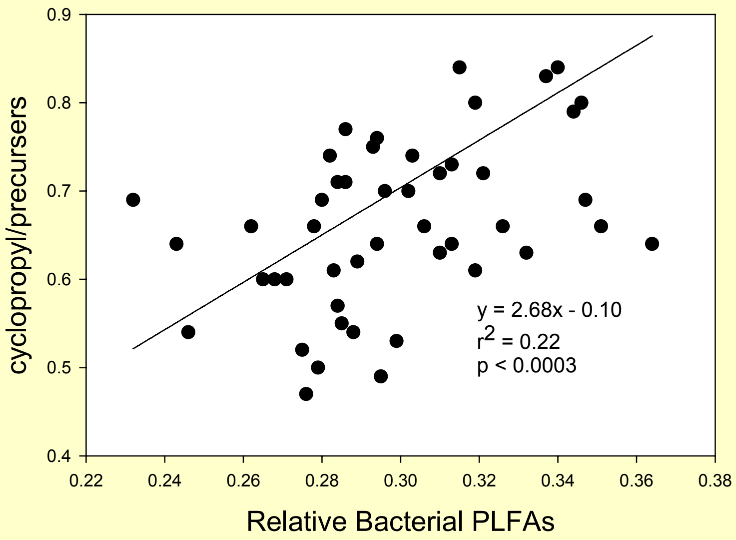
A strong negative relationship for the ratio of AMF PLFA 16:1ω5c with saprophytic fungal PLFA 18:2ω6,9 and the cyclopropyl fatty acid to precursor ratio suggest amelioration of stress is evident for bacteria as the amount of AMF increases.

Fermilab Restoration Chronosequence

The relative amount of AM Fungal PLFA is negatively associated with the cyclopropyl / precursor ratio indicating that as the proportion of AMF fungi increases as a greater proportion of bacterial cells are in log phase growth.

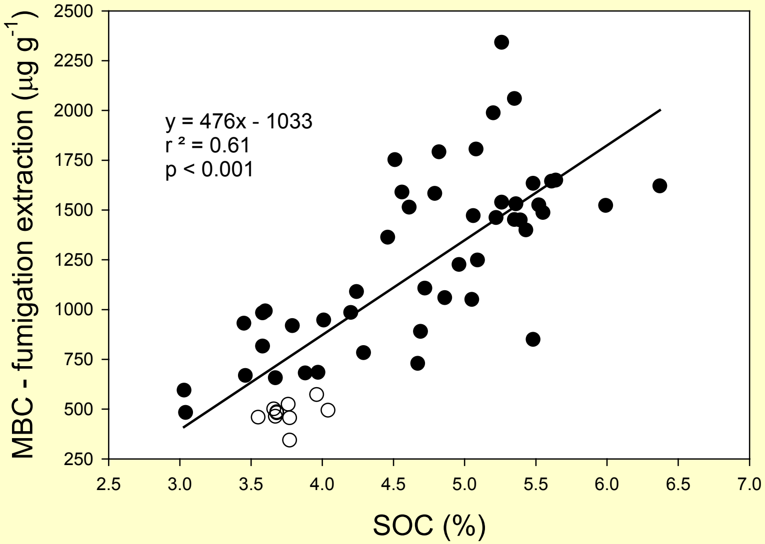


Within the restoration chronosequence the relative proportion of bacterial cells increases with the cyclopropyl to precursor ratio increases indicating as the relative density of bacteria increases a greater proportion of them are in stationary phase growth – an indicator of stress.

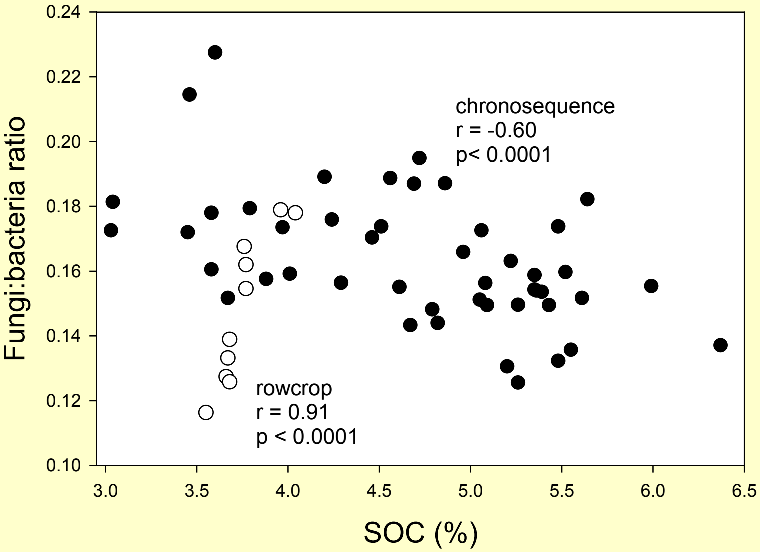


Fermilab Restoration Chronosequence

A positive linear relationship exists between MBC and the amount of SOC along the restoration chronosequence (R^2 = 0.61, p <0.001).

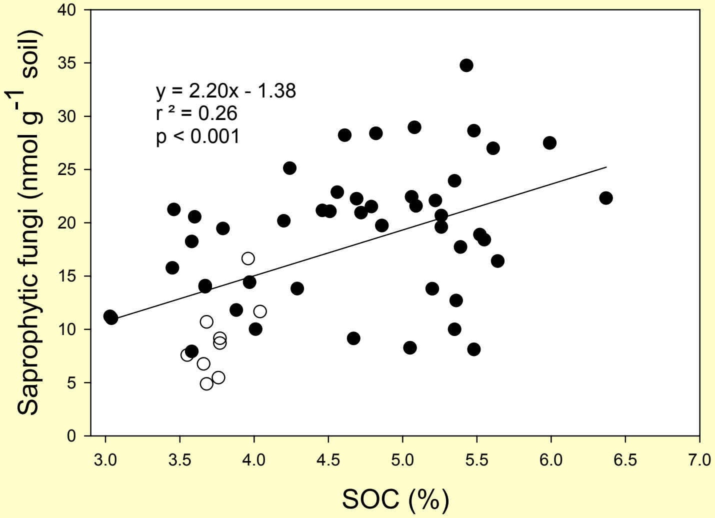


The proportion of fungal-to-bacterial PLFAs shows a positive relationship with SOC in the row crop soils, while decreasing within the chronosequence suggesting that in an aggrading system the amount/activity of saprophytic fungi decrease as C content increases.

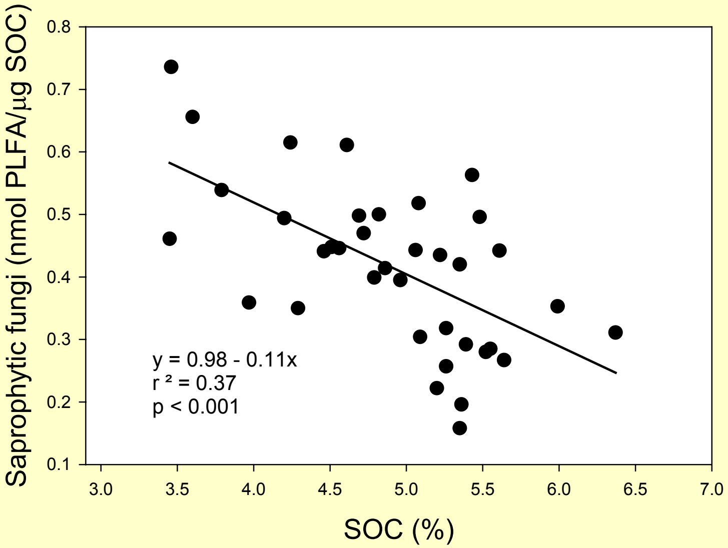


Fermilab Restoration Chronosequence

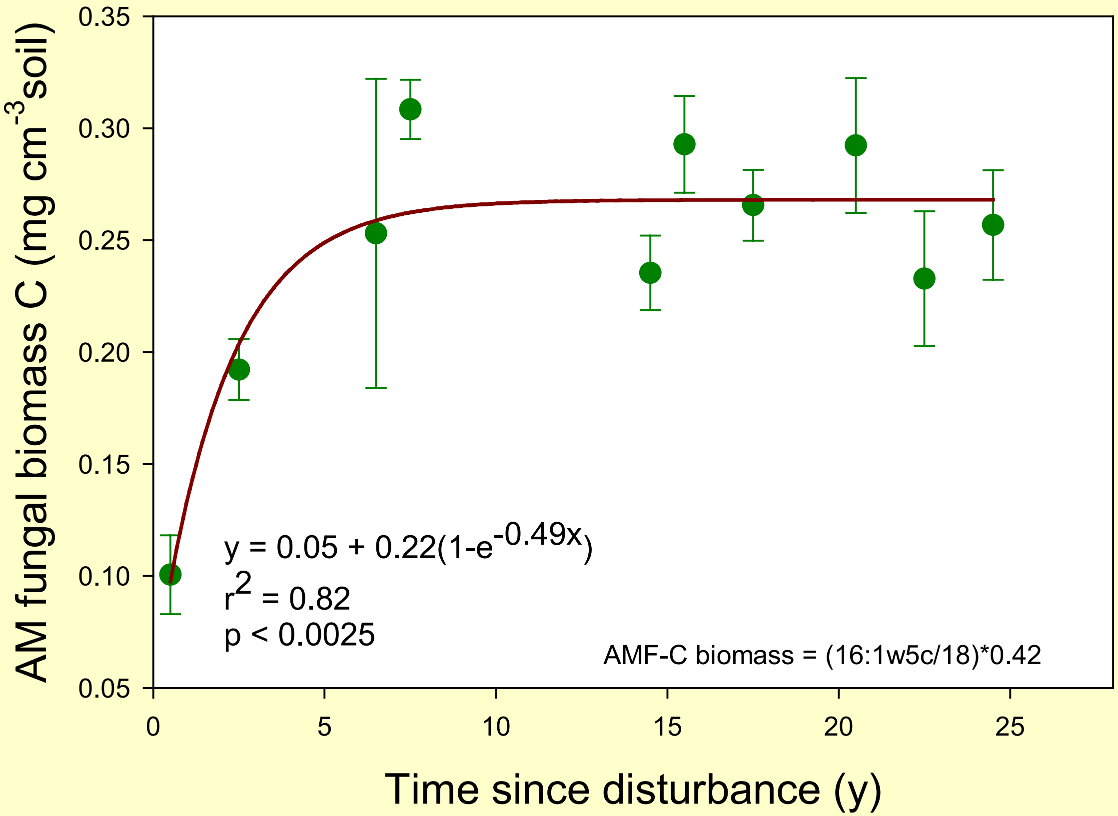
The total amount of saprophytic fungal PLFAs increases as SOC accumulates within the restoration chronosequence. A similar response with SOC also exists for bacterial PLFAs.



If saprophytic fungal PLFA is expressed on a soil carbon basis rather than by soil dry wt a significant negative association exists between saprophytic fungi and SOC . A marginal relationship exists for bacterial PLFAs and SOC (p = 0.072).



The amount of arbuscular mycorrhizal fungi along the chronosequence (0-5 cm depth)

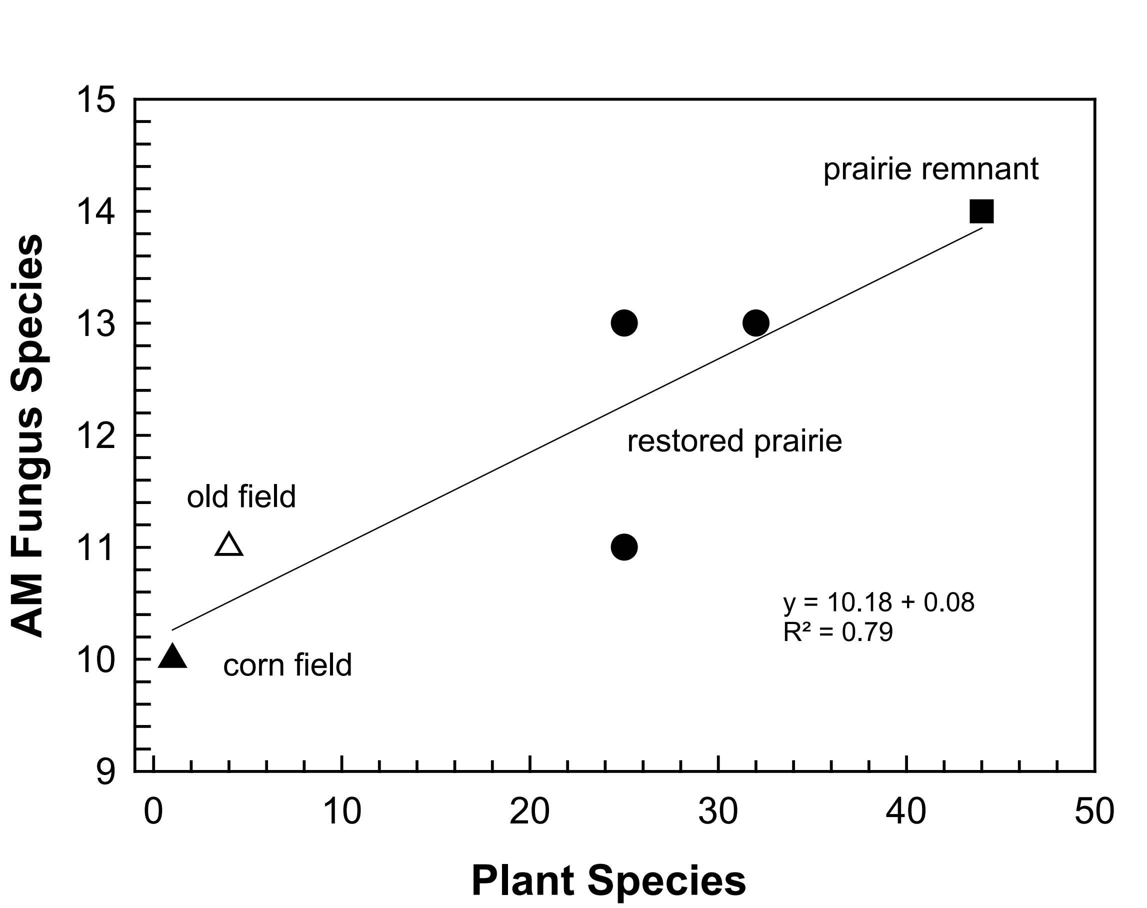


Extraradical hyphae (ERH) of AMF represent a considerable portion of the biomass in soil

In a tallgrass prairie community soil:

- *Peak Extraradical hyphal C (ERH-C) = 215 μg cm^{-3} soil (110 m cm^{-3} soil)*
- *Peak MBC (1068 μg cm^{-3} soil)*
- *Production of ERH-C = 84 μg cm^{-3} soil*
- *ERH-C/MB-C = 0.23*
- *ERH-C turnover* ($T = P/_{Bmax}$) *= 2.42 y*

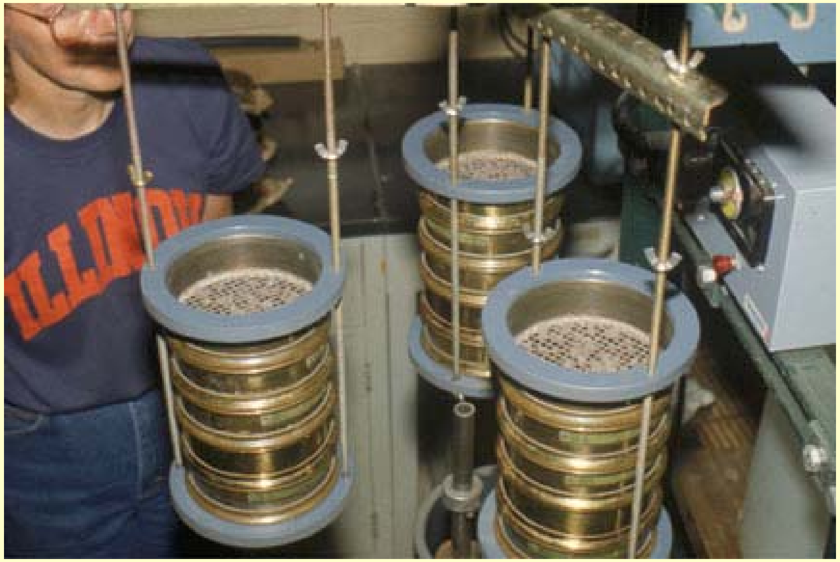
Plant and AMF biodiversity along the prairie chronosequence



Measurements of soil aggregation

Basically the energy of disruption needs to be matched to the soil

- Wet sieving
  - Capillary wetting
  - Fast wetting
  - Misting
- Dry sieving
- Sonication
- Other methods



Nested sieves

Distribution of water stable aggregates affected by land-use practices

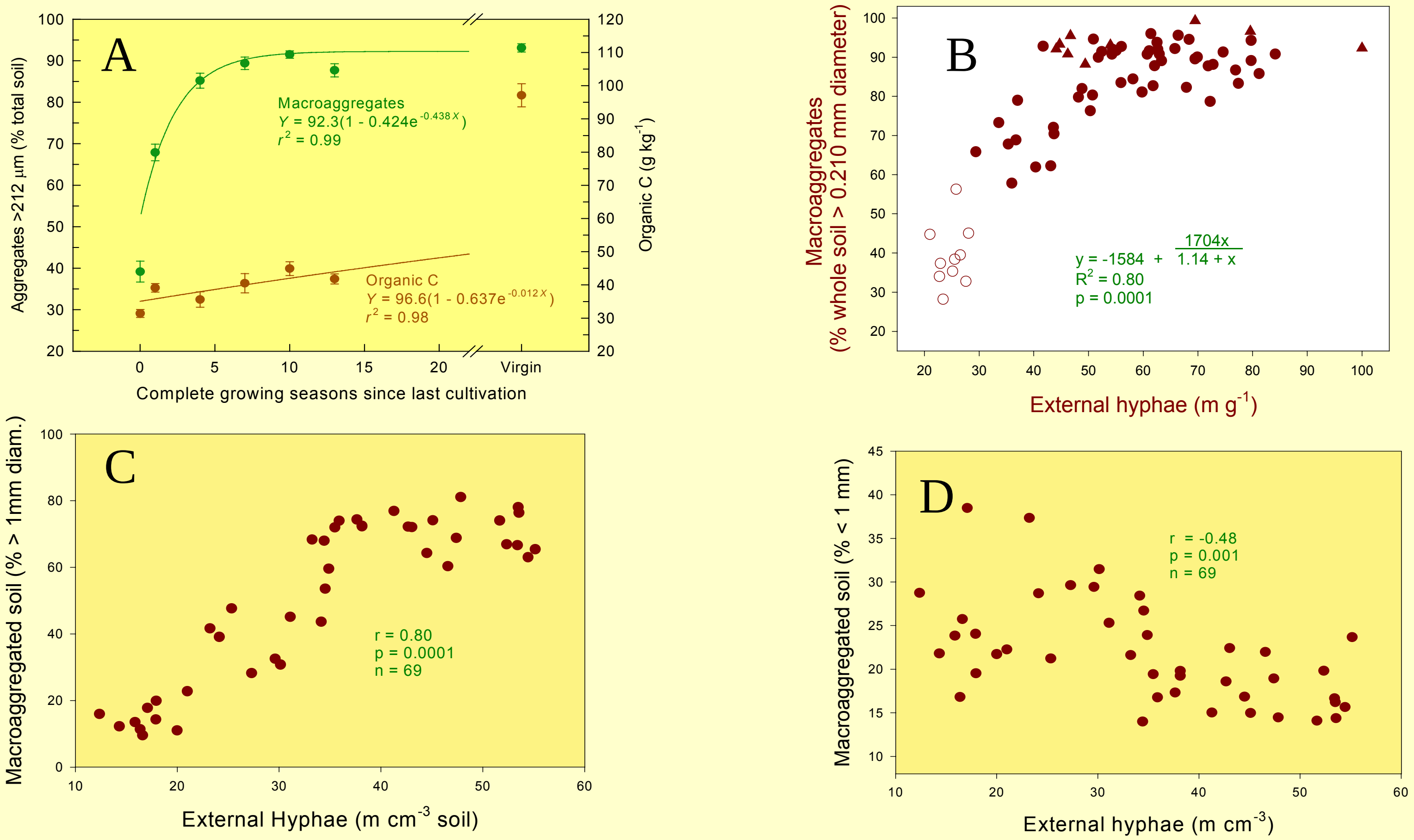
- Continuous cropping results in a greater proportion of water-stable aggregates in smaller size fractions

150 years cultivation

- The return of row-cropped soils to grasslands results in an increase in the proportion of soil held as macroaggregates

12 y restoration

Association soil macroaggregates with soil carbon and external hyphae along the restoration chronosequence



Relationship between macroaggregates (>212 μm) and various biotic factors

Factors	r
Fine root length	0.91***
Very fine root length	0.85***
External hyphal length	0.89***
Microbial biomass	0.65***
Hot-water soluble C	0.55***
Whole soil carbon	0.43***

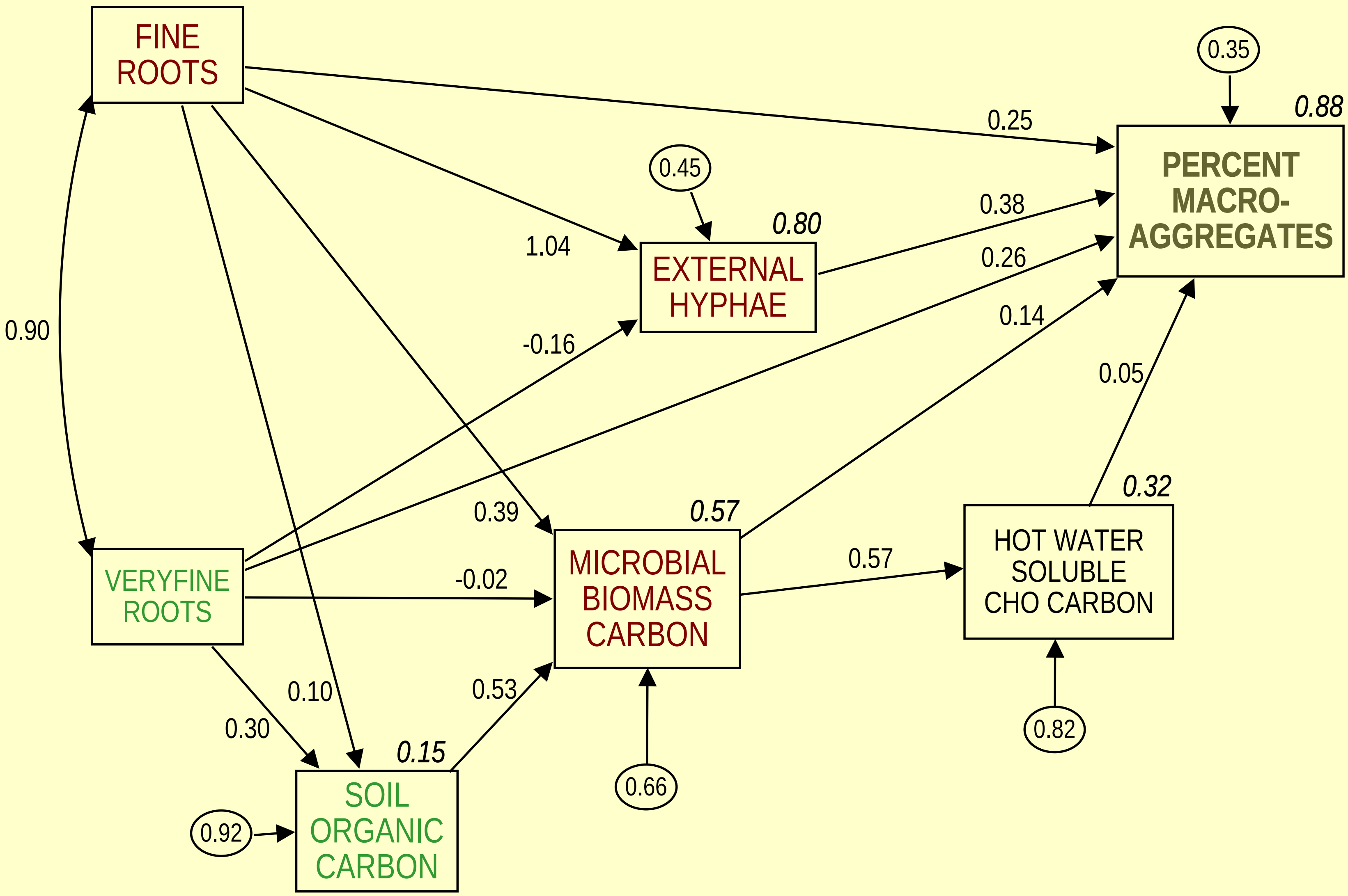
Pearson r coefficients and significance levels: $p<0.0001$***

What to do when everything is significant!

Path Analysis (S. Wright, 1934)

- Path modeling techniques do not allow determination or testing of causality between variables.
- A priori knowledge of the system or theoretical grounds are used to construct a conceptual model referred to as a path diagram consisting of the causal and non-causal interrelationships among measured variables.

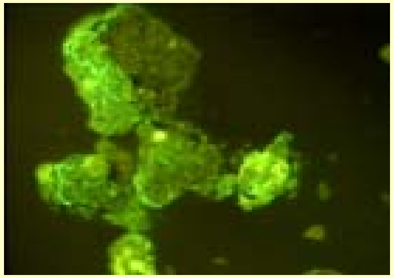
Path diagram for predictors of macroaggregation



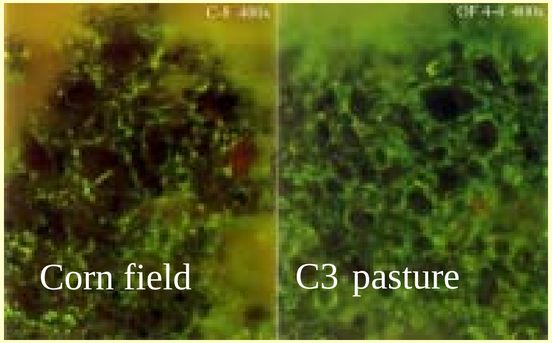
Partitioning of binding agents associated with soil macroaggregates into direct and indirect causal effects based on path analysis (n = 49).

Measured parameter	Direct effect	Indirect effect	Total effect	Correlatio (r)
Fine root length	0.25	0.47	0.72	0.91
Very fine root length	0.26	-0.04	0.22	0.85
External hyphal length	0.38	0	0.38	0.89
Microbial biomass carbon	0.14	0.03	0.17	0.65
Hot-water soluble carbon	0.05	0	0.05	0.55
Soil organic carbon	0	0.09	0.09	0.43

The Glomalin Story



- Mycorrhizal hyphae as a sticky-string-bag
  (*The sticky on the string*)
- Glycoprotein production - Glomalin
  - –stress protein?
  - –hydrophobic nature – desiccant protector
    - •structural integrity
    - •growth across pore space in soil
  - –glue for stabilizing soil aggregates
  - –Contributes to soil carbon and nitrogen pools
  - –Chelater of metals, especially iron, zinc



(Wright & Upadhyaya, Soil Science 161: 575-586, 1996; Wright et al, Plant & Soil 181:193-203, 1996; Rillig et al. Nature 400, 628, 1999; Miller & Jastrow, 2000)

Roots, hyphae, glomalin and soil aggregation – Fermi chronosequence

	SOC	**Clay %**	*Percent macro - aggregates*	*Aggregates <210µm diameter*
Immuno-reactive glomalin	**0.93*****	*0.44***	0.28*	0.19 ns
Extraradical hyphal length	-0.14 ns	0.02 ns	**0.60*****	**-0.50*****
Fibrous root length	*0.33***	0.07ns	**0.61*****	*-0.41***

Pearson r coefficients and significance levels: p<0.001***, p<0.01**, p<0.05*, ns=not significant

The contributions of AM fungi to soil aggregation (*sensu Miller and Jastrow, 1990, 1992, 2000*)

- Physical enmeshment of primary particles and microaggregates
- Localized drying around roots and hyphae
  - Increased physical contact
  - Denaturing and polymerization
- Stabilization of aggregates by cementation
  - Mucigels and glomalin
  - By-products of rhizosphere - supported microbes
- Major source of organic matter inputs
  - Substrate for decomposers (saprophytic fungi, bacteria, soil fauna)
  - Intermicroaggregate binding agent
  - Microaggregate cores

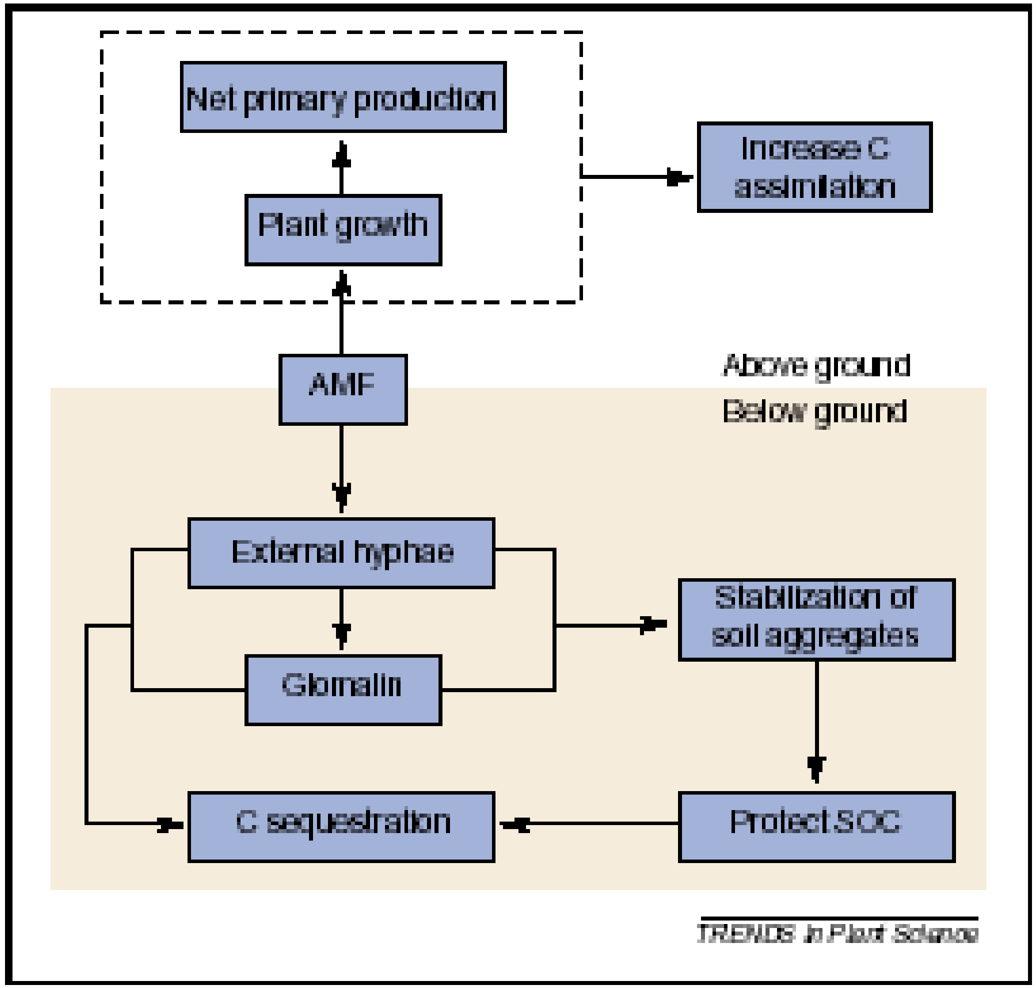


Fig. 1. Role played by arbuscular mycorrhizal fungi (AMF) in regulating carbon fluxes between the biosphere and the atmosphere. Abbreviation: SOC, soil organic carbon.

Zhu & Miller, Trends in Plant Sciences, 2003

Where do we go from here?

- What is the nature, origin, and long-term stability of the C being accumulated in soils of different types?
- How do different management practices affect soil C accumulation and stabilization?
- What are the saturation limits for storing C in various soil types? What factors control these limits?
- Can we model measurable pools that are functionally meaningful and tied to processes?